

BEHAVIOR-BASED ASSESSMENT OF THE AUDITORY ABILITIES OF BRUSHTAIL POSSUMS

MIZUHO OSUGI¹, T. MARY FOSTER¹, WILLIAM TEMPLE¹, AND ALAN POLING²¹UNIVERSITY OF WAIKATO HAMILTON, NZ²WESTERN MICHIGAN UNIVERSITY

Brushtail possums (*Trichosurus vulpecula*) were trained to press a right lever when a tone was presented (a tone-on trial) and a left lever when a tone was not presented (a tone-off trial) to gain access to food. During training the tone was set at 80 dB(A), with a frequency of 0.88 kHz for 3 possums and of 4 kHz for the other 2. Once accuracy was over 90% correct across five consecutive sessions, a test session was conducted where the intensity of the tone was reduced by 8 dB(A) over blocks of 20 trials until accuracy over a block fell below 60%. After each test session, training sessions were reintroduced and continued until accuracy was again over 90%, when another test session was conducted. This process continued until there were at least five test sessions at that tone frequency. The same procedure was then used with frequencies of 0.20, 0.88, 2, 4, 10, 12.5, 15, 20, 30, and 35 kHz. Percentage correct and d' decreased approximately linearly for all possums as tone intensity reduced. Both sets of lines were shallowest at the higher frequencies and steepest at the lower frequencies. Hit and false alarm rates mirrored each other at high frequencies but were asymmetric at lower frequencies. Equal d' contours showed that sensitivity increased from 2 to 15 kHz and continued to be high over 20 to 35 kHz. The possums remained sensitive to the 20 to 35 kHz tones even at low intensities. The present study is the first to report the abilities of possum to detect tones over this range of frequencies and its results support the findings of a microelectrode mapping survey of possums' auditory cortex.

Key words: signal detection, audition, psychophysics, brushtail possums

Brushtail possums (*Trichosurus vulpecula*) are marsupials that are protected in Australia, but a major pest in New Zealand as they are an introduced species that has no natural predators. In New Zealand they threaten the native bird population by decimating the forests and eating birds eggs, and they spread TB to cattle. Understanding the sensorium of pest animals such as possums is useful in developing strategies to control them (Brockie, Fitzgerald, Green, Morris, & Pearson, 1984; Clout & Sarre, 1997; Wynne & McLean, 1999), but very little is known about possums' senses. For instance, only four studies concerned with their sense of hearing have been published (Aitkin, Gates, & Kenyon, 1979; Gates & Aitkin, 1982; Signal, Foster, & Temple, 2001; Winter, 1976).

One approach to studying possums' hearing is to examine the characteristics of the calls that they emit, because it is widely and reasonably assumed that animals call at frequencies and intensities that conspecifics can detect (e.g., Aitkin, 1995; Biggins, 1984; Croft, 1982). Taking this tack, Winter (1976) recorded several calls of brushtail possums, and found that their frequencies were between approximately 0.5 kHz and 12 kHz. He noted, however, that he could not measure frequencies over 12 kHz, thus further research is required to find the full frequency range of possum's calls. To our knowledge, such research has not been conducted.

In 1979, Aitkin et al. published a pioneering anatomical and physiological investigation of possums' auditory system. They reported that possums' cochlea appeared similar to that of other mammals, but in possums a spongy mass of bone, different from the bullae of many eutherian mammals, surrounded the round window. Aitkin et al. (1979) also measured possums' cochlear microphonic potentials (CMP) at the round window. They did so by determining the sound intensities that were required to evoke 50 μ V CMP at frequencies

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Address correspondence to Alan Poling, Department of Psychology, Western Michigan University, Kalamazoo, MI 49008 (e-mail: alan.poling@wmich.edu).

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from 0.1 to 10 kHz. Findings were compared with those for domestic cats, and showed that both possums' and cats' cochleas were sensitive within the 0.1 to 10 kHz range, with the greatest sensitivity being between 0.7 and 1 kHz. The possums' cochlea was more sensitive than the cats' to tones between 0.1 and 0.4 kHz. Aitkin *et al.* (1979) noted, however, that CMP to tones over 10 kHz were not examined in their study, and they recommended further research to study such frequencies.

Subsequently, Gates and Aitkin (1982) conducted a microelectrode mapping survey of possums' auditory cortex. This study measured evoked potentials and the responses of single units and unit clusters to tones that varied in frequency and intensity. Results indicated that the auditory cortex was sensitive to tone frequencies between 330 Hz and 39 kHz, with the greatest sensitivity being between 17 and 19 kHz at 10 dB SPL.

Gates and Aitkin (1982) reported that this pattern of sensitivity is very similar to that observed previously with North American opossums (*Didelphis virginianal*) using a behavioral technique, conditioned suppression (Ravizza, Heffner, & Masterton, 1969). Ravizza *et al.* found that the opossums heard (i.e., reacted to) tones between 0.5 and 64 kHz, with the greatest sensitivity occurring between 16 and 32 kHz, approximately. Thus, the auditory sensitivities of the two marsupial species appear to be roughly comparable. Gates and Aitkin noted, however, that the relation between behavioral and physiological measures of sensory acuity is unclear. For example, even if a given tone caused an evoked potential in the auditory cortex, this does not necessarily mean that the possums could react to (i.e., detect) the tone behaviorally. Therefore, Gates and Aitkin suggested that studies of possums' behavioral reactions to sounds are needed to understand their sense of audition adequately.

Only one previous study of audition in possums has been reported (Signal *et al.*, 2001). It used a two-response conditional-discrimination procedure to examine possums' ability to detect a single frequency, 880 Hz, when presented at varying intensities. Possums were trained initially to press one of two levers when the tone, set at 80 dB(A),

was played (tone-on trials), and to press the other lever when the tone was absent (tone-off trials). Once the possums had learned this discrimination, test sessions were conducted. These started with the tone training intensity and the intensity was then reduced after a block of trials provided the possum responded at or over 60% correct in that block. Sound intensity was reduced until the possums responded below 60% correct on a block of trials. At this point, there was a block of trials at the training intensity. The possums were able to discriminate the 880 Hz tone well at 80 dB(A), and their ability to discriminate it deteriorated roughly linearly as the tone intensity decreased. Signal *et al.* extrapolated from their data to suggest that, on average, their possums could detect the sound until it reached approximately 36 dB(A).

Because only one frequency, 880 Hz, was investigated by Signal *et al.* (2001), further behavioral research is needed to develop a full picture of possums' auditory ability. Signal *et al.*'s orderly findings suggest that their procedure could be an appropriate one for gathering additional behavioral data. The purpose of the present research was to assess the auditory abilities of brushtail possums at a variety of tone frequencies, employing the behavior-analytic procedure used by Signal *et al.*, to measure their relative sensitivity to different frequencies within the context of their home room.

Data analysis in the present study followed that of Wright (1974), who plotted relative sensitivity functions for hue discriminations in pigeons. One type of function he discusses is based around equal d' contours. Here, d' is a measure of sensitivity suggested by Signal Detection Theory (SDT; Green & Swets, 1966) that can be derived from the type of data obtained from the method used by Signal *et al.* (2001). Wright derived his equal d' contours from the psychometric function relating d' to wavelength difference at each wavelength tested. This involved finding the wavelength difference associated with a particular d' for each frequency then plotting these values against frequency for each d' value. This gave a family of functions that illustrated the animals' sensitivity in the experimental context over the wavelengths tested.

The aim of the present study was to produce such functions for auditory stimuli with possums. These functions are similar to an audiogram in which the minimum perceptible intensity (often that required to detect the stimulus 50% or 75% of the time) is plotted against different frequencies of the sounds in Hz (e.g., Aitkin, Bush, & Gates, 1978; Aitkin et al., 1979; Ravizza et al., 1969; Temple, Foster & O'Donnell, 1984). Producing equal d' contours requires the same experimental manipulations as producing an audiogram, but the former illustrates performance over the whole range of intensities. Such functions are somewhat time intensive to generate, as are audiograms, but they provide an excellent summary of a species' (or an individual's) sense of hearing within the context studied (Temple et al., 1984; Wright, 1974).

METHOD

Subjects

Five mature brushtail possums, 1 female (Caper) and 4 males (Wembley, Peppi, Mickey, and Mouse), served as subjects. At the beginning of the study, their approximate ages were 1.5, 1, 3, 3.5, and 3.5 year(s), respectively. Wembley and Peppi had been given to our research facility as babies after being found on their mothers, who had been shot, and both were hand reared. Mickey and Caper were born in our colony and reared by their mothers, and Mouse was captured from the wild as a young adult. Four animals were experimentally naïve. Mickey had previous experiences in performing on visual discrimination tasks and under simple fixed-ratio and concurrent schedules of food reinforcement.

The possums were kept in individual cages in a colony room under a 12-hr/12-hr light/dark cycle. The temperature was kept between 15 and 25 °C; heating and cooling were used only when temperatures in the building moved outside these values, otherwise the room was at the ambient temperature for the time of year. Supplementary feed of dock leaves and apples or carrots were given to every possum daily after the experimental session finished. Possum food pellets, manufactured by Camtech Nutrition Ltd. (Hamilton, New Zealand), were provided in the morning when no experimen-

tal session was conducted. Possums were weighed every 2 weeks, and the amount of food given to each animal outside experimental sessions was adjusted accordingly to maintain a steady weight. Water was available to each possum at all times. The methods of animal husbandry used for these experiments have been outlined by Signal, Foster, Temple, and Chandler (2005). The research methods were reviewed by and received ethical approval from the University of Waikato Animal Ethics Committee.

Apparatus

Experimental sessions were conducted in the possums' home cages (860 mm long, 510 mm wide, and 540 mm high). A wire shelf was located 250 mm from the top of each cage, and a plywood nest box (355 mm long, 455 mm wide, and 200 mm high) was attached to the top of each cage on the outside. Each possum had access to its nest box (from the cage) through an aperture (220 mm \times 120 mm). A plywood door (550 mm \times 330 mm) containing the response panel was attached to the front of each cage. A magazine attached to the center of the response panel could be raised to allow the possum access to food through a hole (30 mm \times 30 mm). Magazines for all possums contained a mixture of steamed flaked barley and Cocoa Puffs cereal (Budget/Safeway, Auckland, New Zealand) mixed at a ratio of 15:1 by volume.

Above the magazine aperture each response panel contained three holes (each 6 mm in diameter) through which response levers (250 mm in length) could be inserted. The minimum force required to operate each of the levers was 0.25 N. An amber light (2.1 V, 590 nm) was located 50 mm above each lever. A Flying Lead Piezo Sounder (ABI 006-Piezo Indicator, RS Components, Kwai Chung, Hong Kong) measuring 20 mm in diameter was attached to the back of the response panel, 9 cm from the left side and 50 mm from the top of the response panel. The sounder provided possums with auditory feedback, a 0.05-s beep [90 dB(A), 3.7 kHz], immediately following each correct response (see below). The possums' cages were located in a row along a wall in a 2-m wide room where the background noise from the possums moving around in their cages varied between 28.5

and 50 dB(A).¹ The peaks in noise intensity came when a possum moved around in its cage; when possums were still the steady background noise was at the lower end of this range. The cages faced a blank concrete block wall located 1.2 m from their front panels.

Two different types of speakers (Digitor indoor/outdoor speakers; A9772—DSE Ltd, Auckland; and Foster ribbon tweeters—Foster Electric, Tokyo) were used to play auditory stimuli at different frequencies. The Digitor speakers were used to produce sounds between 200 Hz and 10 kHz; Digitor speakers modified by detaching the internal woofers were used to play tones between 12.5 and 15 kHz; and the tweeters alone were used to produce tones between 15 and 35 kHz. Either the speaker or the tweeter was set 100 mm above the central light.

An oscilloscope (Kikusui 40 MHz COS 5040, Kikusui Electronics, Yokohama, JN) a multi-meter (Fluke 79 series, Fluke Corporation, Everett, WA, US), and a microphone with a frequency range between 20 and 20,000 Hz (Sennheiser 21/22, Sennheiser Electronic, Wennebostel, DL) or, for frequencies above

20 kHz, a tweeter, used as a microphone, were used to test each tone frequency to make sure each speaker was producing the desired tones and the desired intensities. To test the frequencies, the microphone or tweeter was placed roughly where the possum's head would be during responding and the wave forms received were examined to check for interference. If a frequency did not produce an appropriate waveform then it was not used. It was found that 100 Hz and some of the frequencies lower than this resulted in interference in this situation and so 200 Hz was the lowest frequency used.

A MED PC programmable audio generator (ADSL, ANL-926) controlled the frequency and loudness of tones (Med Associates, St. Albans, VT, US). The specifications of the programmable audio generator allowed for production of tones between 10 and 35,000 Hz with a range of volume between 20 and 100 dB(A). A sound pressure meter that could measure from 30 to 130 dB(A) for tones between 0.1 to 25 kHz (Testo 816, Testo, Victoria, AU) was used to assess tone intensity. For tones of 30 and 35 kHz, where the sound pressure meter could not measure the intensities, the training intensity was set with the tone frequency at 7 kHz, and then the frequency was changed to 30 or 35 kHz. The oscilloscope was used to check the amplitude of the signal generated by each tone frequency at each intensity used. The data obtained [the logarithms of average voltage (reported as the root mean square: RMS)] are shown plotted against the programmed dB(A) level [for intensities of 48 dB(A) or above] in Figure 1. The top panel (A) shows the data from the 200 Hz to 20 kHz frequencies where the intensity could be measured with the sound pressure meter. These data show that the logarithms of the average voltage was the same across any dB(A) level for all frequencies. Thus, the voltage measures correlated with the measured sound pressure levels. The second panel (B) shows the data from the 20 kHz tone when the sound intensity was set in two ways: using the sound pressure meter and using the calibration method (from a 7 kHz tone). The figure shows similar data from both methods, suggesting the calibration method was accurate. The third panel (C) shows the data from the 30 and 35 kHz tones where the intensities were set using the calibration method. The

¹ Sound pressure is normally measured in decibels (dB). Decibels are defined as 10 times the logarithm of the ratio of the signal sound pressure level to a reference sound pressure level. Human ears are more sensitive to sounds between 1 to 4 kHz than they are to sounds at very low or very high frequencies and this means that small changes in intensity have bigger perceptual effects within the sensitive range. Thus, commercially available sound pressure meters have built-in filters that adapt the received sound pressure to a measure that follows the human sense of sound rather than reporting the received sound pressure directly. The two most frequently used filters give the intensity measure as dB(A) and dB(C), with dB(A) the more common. Under the decibel A filter a particular size change in measured sound pressure has less effect on the resulting dB(A) at high and low frequencies than on the resulting dB(A) for frequencies in the 1–4 kHz range (see <http://www.animations.physics.unsw.edu.au/jw/dB.htm> for further information). The dB(A) scale generally resembles the inverse of the 40 dB (at 1 kHz) equal-loudness curve for the human ear. Readings in dB and dB(A) at 1 kHz will be the same. The web site found at <http://www.sengpielaudio.com/calculator-dba-spl.htm> provides a calculator which gives the weightings that are applied in reporting dB(A) for a selected frequency and the formula used in converting from dB to dB(A). The sound pressure meter employed in the present study used the decibel A filter, as do most commercially available meters, and so provided measures in that scale. Because there is no simple way to convert the reading from dB(A) to dB, we report measures as provided by our meter, i.e., as dB(A).

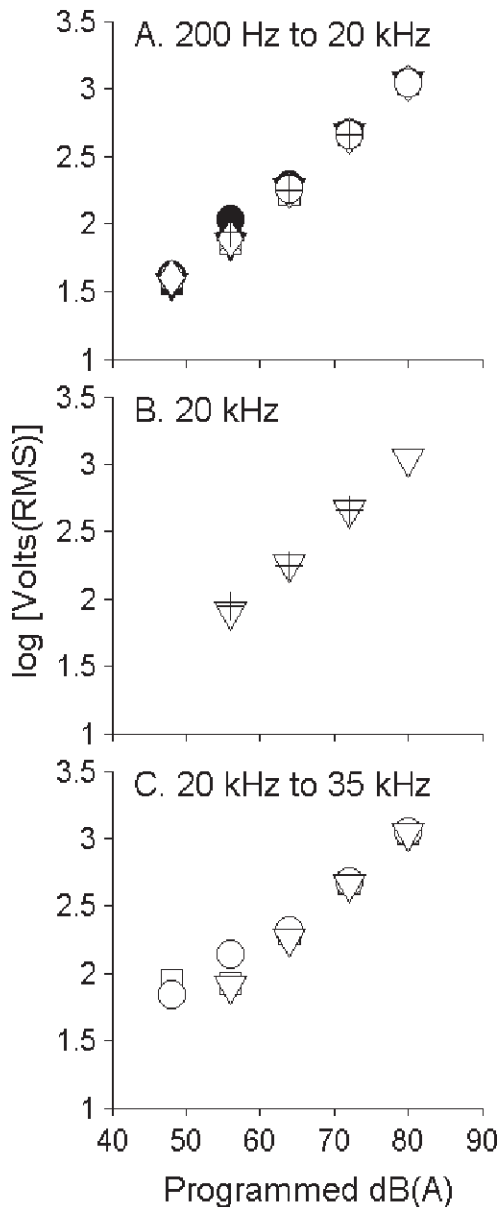


Fig. 1. The logarithms of the voltage (RMS) measured by the oscilloscope for each frequency plotted against the intensity [dB(A)].

A. The data from the 200 Hz to 20 kHz tones (200 Hz = open circles, 880 Hz = filled triangles, 2 kHz = filled squares, 4 kHz = open squares, 10 kHz = asterisks, 12.5 kHz = open diamonds, 15 kHz = open circles, 20 kHz = pluses). In all cases the training intensity was set using the sound pressure meter and the test intensities were set by the MED PC programmable audio generator which was used to decrease the intensity of the tones over the test sessions.

B. The data from the 20 kHz tone. The pluses show the data when the initial intensity was set using the sound

data from the 20 kHz tone with the intensity set by the same method are shown for comparison. For the 30 and 35 kHz tones the voltages at the various intensities are similar to those from the 20 kHz tone at the same intensities. This suggests that the sound pressure levels set by calibration for the 30 and 35 kHz stimuli were accurate.

The programmable audio generator initially operated a public address amplifier (Toa Electronics, Burlingame, CA, US); this device failed and was replaced (during the 15 kHz condition for Wembley, Peppi, and Mouse; and the 20 kHz condition for Caper and Mickey) with a Yamaha AX-890 amplifier (Yamaha Corporation, Shizuoka, JN). The amplifier was connected through a latching relay to the speakers so as to be able to produce sound from one speaker at a time. The relay was situated next to the experimental control equipment and computer, which were located in separate room from the possum, and the relay operated only at the start of each session when the speaker for that possum was selected. Therefore, it is unlikely that possums heard the relay operate and impossible that its sound, if heard, served a discriminative function.

Experimental events and data recording were controlled by a computer (Dell Optiplex, Round Rock, TX, US) using MED PC TM IV software and ME PC interfaces.

Procedure

A session was conducted for each possum 5 to 7 mornings a week, with the first possum's session starting approximately 2 hr into the possums' dark cycle. All possums were exposed to a series of training and testing conditions. There were three different training conditions: lever-press training, and side-lever training with and without prompts. Caper, Mickey, and Mouse were trained with a tone frequency of 880 Hz, while Peppi and Wembley were

← pressure meter (as in A) and the open triangles show the data when the initial intensity was set from 7 kHz by the calibration method.

C. The data from the 30 (open circles) and 35 (open squares) kHz tones with the initial intensity set from 7 kHz by the calibration method. The data from the 20 kHz tone with the intensity set by the same method (open triangles as in B) are shown for comparison.

trained with a 4 kHz tone. Each training session lasted until 100 food deliveries occurred or 40 min passed, whichever happened first. Training began for Caper, Mickey, and Mouse before it was initiated for the other two animals.

Lever-press training. At the beginning of each session, only the center lever was inserted and the light above that lever (center light) was illuminated. Every lever press provided auditory feedback, darkened the center light, and provided 3-s access to food (the reinforcer), followed immediately by a 3-s intertrial interval (ITI). During the ITI the center light was turned off. Delivery of a reinforcer after every response constitutes a fixed-ratio (FR) 1 schedule of reinforcement. Mickey, who had previous experimental experience, quickly began responding under this schedule. The 4 experimentally naïve possums did not do so and so reinforcement of successive approximations (shaping) was used to establish lever pressing (Signal *et al.*, 2001). Once all possums were operating the lever consistently (i.e., 100 times within 40 min for at least three consecutive sessions), side-lever training with prompts began. Under all training and testing conditions sessions were conducted sequentially across possums with one possum in a session at a time. When one possum completed a session the next possum's session started.

Side-lever training with prompts. Under this procedure, all three levers were inserted at the beginning of each session. In the first phase of this training, there were left- and right-light trials, and no tone was presented. Each trial type was presented pseudorandomly using a version of the Gellerman (1933) series, ensuring there would be approximately equal numbers of left and right trials in a session and no more than three consecutive trials of one type. At the beginning of either trial type the center light was lit and one response on the center lever resulted in the darkening of the center light and the illumination of one of the side lights. A response to the side lever under the illuminated light resulted in a feedback beep. Correct responses were reinforced with food delivered under a variable-ratio (VR) 1.5 schedule of reinforcement. Under this schedule the number of correct responses (i.e., correct trials) required to produce food was, on average, 1.5. The number of correct trials required before the

next reinforcer was selected after each reinforcer, without replacement, from a list of 15 whole numbers derived from an arithmetic series (a , $a + b$, $a + 2b$...) with $a = 1$ and $b = 2$, that produced the sum of 225, i.e., had a mean of 15. The next ratio in the series was derived from the selected number multiplied by 0.1, which was then rounded up to the next whole number. This rounding meant that the actual average ratio with this VR was slightly greater than 1.5. Reinforcer delivery was followed by a 3-s ITI with all three lights off. A response to the side lever under the unlit light resulted in the offset of the other side light, no feedback beep, and a 6-s period with all lights out before the next trial began.

The second phase of this training (five sessions) involved the introduction of tone-on and tone-off trials. The procedure remained the same except that on approximately half of the trials, selected pseudorandomly, a response on the center lever produced a tone [880 Hz at 80 dB(A)] that stayed on until one of the side levers was pressed. Right-lever responses were designated as correct on tone-on trials (and only the light above the right lever was lit following a center-lever response on such trials) and left-lever responses were designated as correct in tone-off trials (and only the light above that lever was lit following a center-lever response on these trials). Responses on the side lever without a light on were designated as incorrect. Correct responses were reinforced with food delivered under the VR 1.5 schedule. Incorrect responses had the same consequences as pressing the lever below an unlit light in the immediately prior phase. If no response was made on a side lever within 1 min of the preceding center-lever response the trial terminated (i.e., all lights were turned off and on tone-on trials the sound was terminated), and a 6-s ITI followed, with all lights off, before the next trial began.

Side-lever training without prompts. This phase was very similar to side lever training with prompts. The difference was that in training without prompts both the left and the right side lights were lit following a response to the center lever during both tone-on and tone-off trials. In addition, over 20 sessions the VR schedule was increased from VR 1.5 to VR 2.5, then finally to VR 4.6 (using the same number series as for the VR 1.5 but with different multipliers).

As noted previously, training began for Caper, Mickey, and Mouse before it was initiated for the other 2 animals. During this phase, Caper and Mouse were accurate on over 90% of the trials. Mickey's accuracy, however, was low. Analysis of the data showed that Mickey had very short latencies to respond on a side lever after a center-lever response, compared to those of the other 3 possums. This meant that he had only very brief tone and no-tone periods. To increase the duration of the stimulus presentation, an FR 5 schedule was introduced on the center lever for all possums. While the FR 5 was being completed on the center lever, the center light was illuminated and during tone-on trials the tone was on. The fifth lever press on the center lever turned off the center light and illuminated the side lights. Responding on either of the side levers turned off the side lights and terminated tone presentation on tone-on trials. Once the FR 5 schedule was introduced, Mickey's accuracy increased to 90% or above, while the accuracy of Caper and Mouse did not change. At this point, training was deemed to be complete. An FR 5 schedule was arranged on the center lever from the beginning of the final phase of training for Wembley and Peppi, whose accuracy exceeded 90%.

Testing conditions. Each experimental condition consisted of two different types of sessions. Some, termed training sessions, were arranged like those in the final phase of the training procedure (although with different tone frequencies), and others were test sessions. At the beginning of each experimental condition, the tone frequency was changed to that required for the condition with the volume set at 80 dB(A) [or 72 dB(A) for 15 kHz and for the first 20 kHz condition] for tone-on trials. Training sessions were conducted until a possum achieved 90% or more correct for 5 consecutive sessions or, if the possum did not achieve this criterion over 30 sessions, the criterion was reduced to either 85% or more correct for 5 consecutive sessions or no visual upward trend in percent correct over 16 sessions, whichever occurred first. When the training was completed test sessions were started, at least five of which were conducted for each possum under every condition.

Each test session contained multiple blocks of 20 trials, in each of which 10 tone-on and 10

tone-off trials were arranged pseudorandomly using a version of the Gellerman (1933) series to ensure there were no more than 3 consecutive trials of any one type. A test session started with a block in which the tone was set at 80 dB(A) [or at 72 dB(A) for 15 kHz and the first 20 kHz condition] for the tone-on trials. If the possum achieved 60% correct over a block of trials, the audio generator was programmed to reduce the volume by 8 dB(A) for the next block of trials. The volume was reduced across blocks until the 60% criterion was not met, or until 24 dB(A) was reached, whichever occurred first. A final block of 20 trials at the initial tone intensity then followed, after which the session ended. If a test session had continued for 60 min, and if the possum had not responded for 20 consecutive min, then the session was terminated at this point. If the possum had responded within the last 20 min, the session continued until there was 20 min without a response. A test session was termed successful when it took less than 60 min and when the possum achieved 80% or more correct in both the first and the last block of trials [i.e., those at the training dB(A)].

At least one training session was conducted after every test session. If the percentage of correct responses in this training session reached the criterion level used for test sessions to be started in that condition, the next session was a test session. If the criterion was not met on the first training session, training sessions continued until it was met, and the next session was a test session. A possum progressed to the next condition when it had completed at least five successful test sessions. Table 1 shows the order of the experimental conditions and the tone frequencies for each possum.

Replication of conditions. The 880 Hz condition was repeated for Caper and the 2 kHz tone was repeated for Mickey and Mouse. During the 15 kHz condition, either a modified speaker or a tweeter was used for different possums. The tweeters were used for Mouse, as these were available at the beginning of his 15 kHz condition. To make sure performance was not affected by the type of speakers used, the 15 kHz condition was repeated with Wembley and Peppi using both the modified speakers and the tweeters. The first 20 kHz condition used 72 dB(A), set using the sound pressure meter, as the training intensity the

Table 1
The order of experimental conditions with each frequency (kHz) for each possum.

Cond.No.	Possum				
	Caper	Wembley	Peppi	Mickey	Mouse
1	0.88	4.0	4.0	0.88	0.88
2	2.0	10.0	10.0	2.0	2.0
3	4.0	0.88	0.88	4.0	4.0
4	10.0	2.0	2.0	10.0	10.0
5	0.88 ^R	12.5	12.5	2.0 ^R	2.0 ^R
6	12.5	15.0	15.0	12.5	12.5
7	15.0	15.0 ^R	15.0 ^R	15.0	15.0
8	30.0	20.0	20.0	20.0	20.0
9	0.2	0.2	0.2	0.2	0.2
10	20.0 ^C	20.0 ^C	20.0 ^C	20.0 ^C	20.0 ^C
11	30.0	30.0	30.0	30.0	30.0
12	35.0	35.0	35.0	35.0	35.0

Note. The superscript R indicates a repeated condition and C indicates the repeat of the 20 kHz condition using the calibrating procedure to set the dB(A) level. See text for details of the replications. The first condition was also the training frequency for that possum.

second (C) used 80 dB(A) set using the calibrating procedure.

RESULTS

All possums completed at least five test sessions with all the frequencies they experienced, with the exception of Mouse in the replication of the 2 kHz tone. Figures 2 and 3 illustrate the performance of each possum at all frequencies and intensities. In these figures, the mean percent correct at each tone intensity averaged over test sessions is plotted against the programmed tone intensity [dB(A)]. Where a condition was replicated the data are included in these means because performance did not differ substantially in the two exposures. The data shown are from blocks of trials where the programmed dB(A) level was above the background noise level [48 dB(A) or above]. The vertical lines around each data point indicate one standard deviation (taken over the data from all successful test sessions for that frequency and intensity for that possum) each side of the means. Figure 4 shows the means of the data across all possums for each frequency and the straight lines fitted to these data.

The figures show that the possums discriminated all tone frequencies well at and near the training tone intensity and that their accuracy generally declined as the intensity decreased. The general trends are similar for all possums with steeply falling data at lower frequencies

and shallower data trends at the higher frequencies. At 15 and 20 kHz the possums were generally responding accurately (close to or above 75% correct) even at 48 dB(A). Figure 4 and Table 2 show the straight lines describe the data well (SE between 1.36 and 4.36 and VAC > 83% in all cases). The data slope most steeply at the lower frequencies, with 2 kHz giving the steepest fitted line; the fitted lines are less steep at the higher frequencies with 30 kHz giving the least steep line. Generally, the intercepts are larger for the higher frequencies.

Signal detection analysis. The probability of a false alarm [P(FA)], that is, of an incorrect response on a tone-off trial, is a measure of the error rate when the tone is absent, and the probability of a hit [P(Hit)], that is, of a correct response on a tone-on trial, is a measure of accuracy when the tone is present. Figure 5 shows these two measures calculated from the data averaged across possums for each frequency. This figure shows that the hit rate increases and false alarm rate decreases as intensity increases for each frequency. As the frequency increases the data paths change. Generally, the hit rate at the lower intensities gets larger, that is, the possums become more accurate on tone-on trials, while the hit rate at the training intensity stays the same. Thus the data path for hits is shallower the higher the frequency. The false alarm rate at the lower intensities tends to decrease as frequency increases, but only to a small degree. The

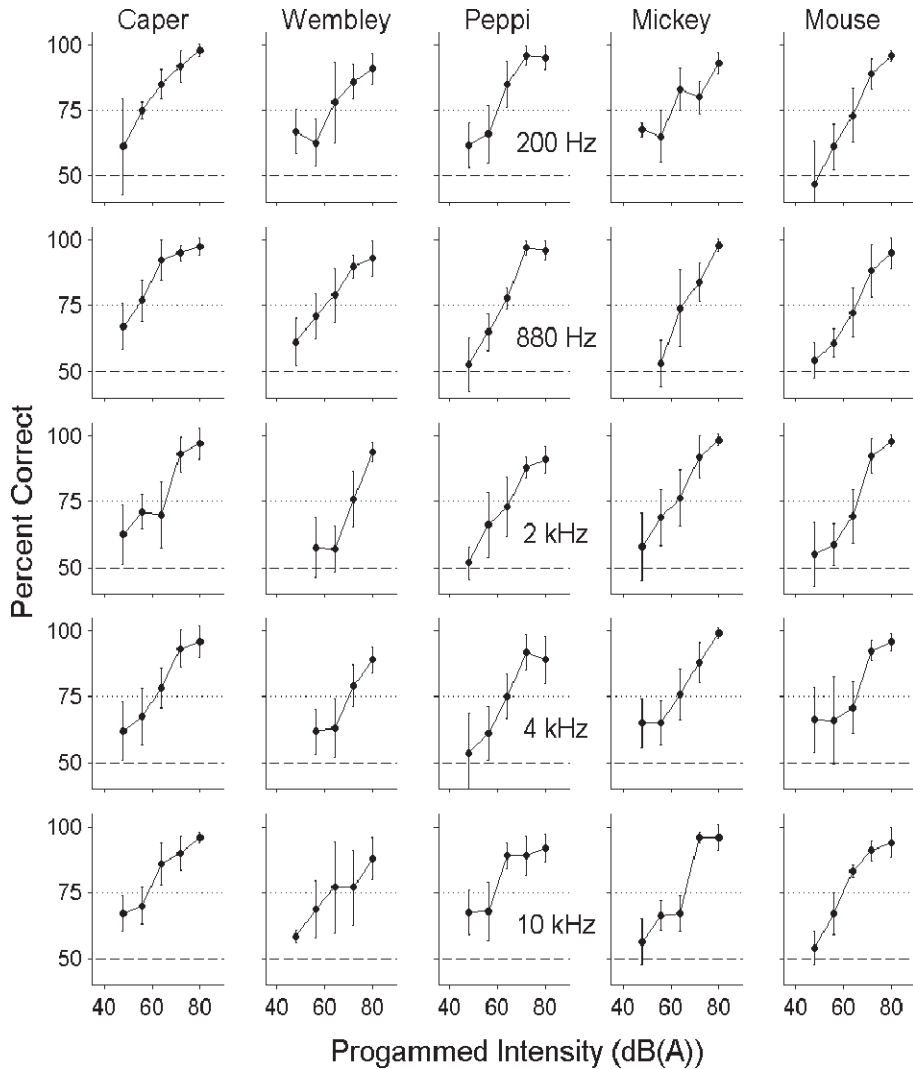


Fig. 2. The mean percent correct for the test sessions with 200 and 880 Hz and with 2, 4 and 10 kHz tones, plotted as functions of the programmed tone intensity for the intensities greater than or equal to 48 dB(A). The vertical lines indicate one standard deviation each side of the data point.

results of these changes is that the two data paths mirror each other more at the higher frequencies than they do at the lower frequencies.

The probabilities of hits and false alarms can be used to derive d' (d-prime), the SDT measure of discrimination. Figure 6 shows d' , calculated as the difference between $z(\text{Hit})$ and $z(\text{FA})$, based on the means shown in Figure 5, plotted against intensity for each frequency. Table 2 shows that the data are well described by the fitted lines (small SE and VAC > 90% in all cases). As with percent correct

(Figure 4) the lines are shallower and the intercepts tend to be larger for the higher frequencies.

Relative sensitivity functions, based on equal d' contours obtained as suggested by Wright (1974), were produced by calculating the intensities equivalent to various d' values for each frequency (derived from the lines in Figure 6 and Table 2). They are shown in Figure 7. Wright (1974) pointed out that each of the equal d' contours shown can be considered a plot of a threshold. That is, each contour reflects the animals' relative sensitivity

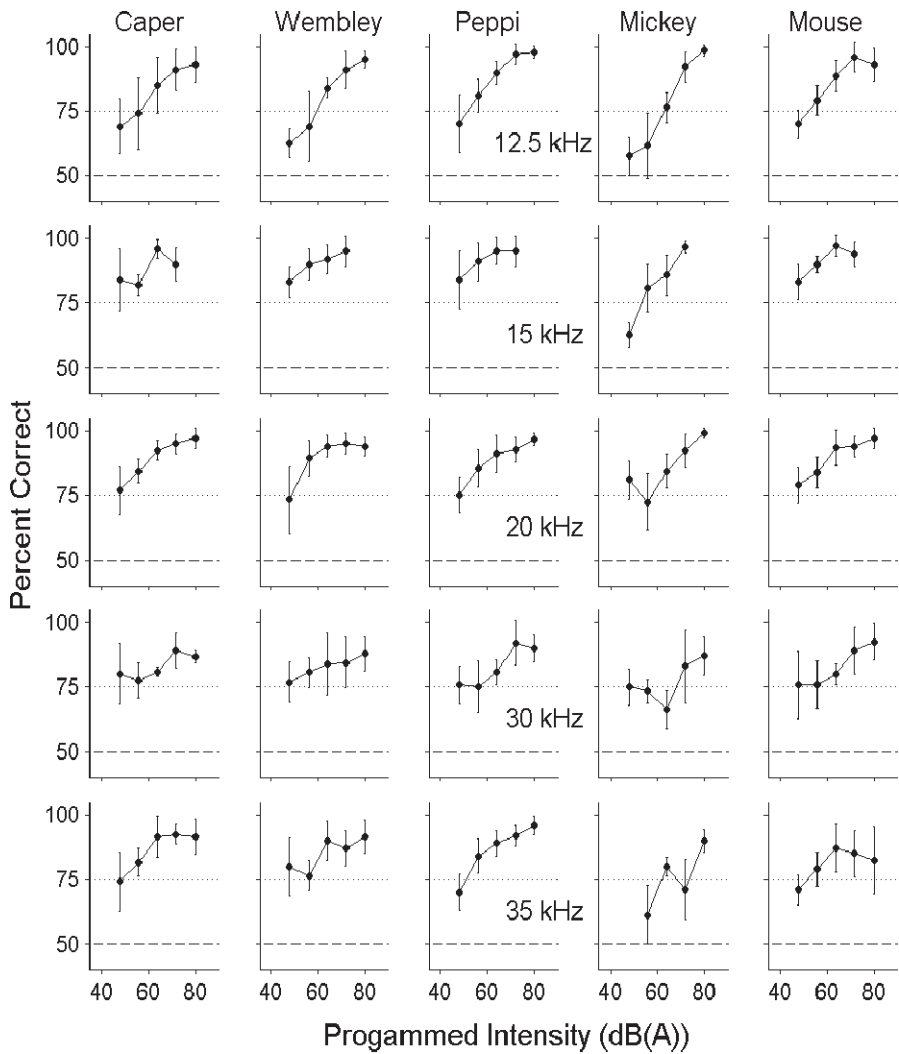


Fig. 3. The mean percent correct for the test sessions with 12.5, 15, 20, 30 and 35 kHz, plotted as functions of the programmed tone intensity for the intensities greater than or equal to 48 dB(A). The vertical lines indicate one standard deviation each side of the data point.

to that intensity over those frequencies in that situation. The steeper the psychometric function relating d' to intensity at a frequency the closer the contours will be at that frequency. The present functions show relatively constant sensitivity over the lowest frequencies up to around 1 kHz. After this sensitivity to the lower intensities increases and reaches its maximum at around 2 kHz (i.e., the functions show the smaller d' values are associated with lower intensities), sensitivity then increases over 2 to 15 kHz and continues to be high over 20 to 35 kHz. The functions suggest that the animals

can detect high frequency tones at low intensities quite well. It should be noted that the scale on the x-axis is quite large and so changes over the 200 Hz to 10 kHz range are somewhat difficult to assess.

DISCUSSION

The purpose of the current study was to measure the auditory acuity of possums and produce a behavior-based relative sensitivity function from equal d' contours. Acuity was assessed at various frequencies by systematical-

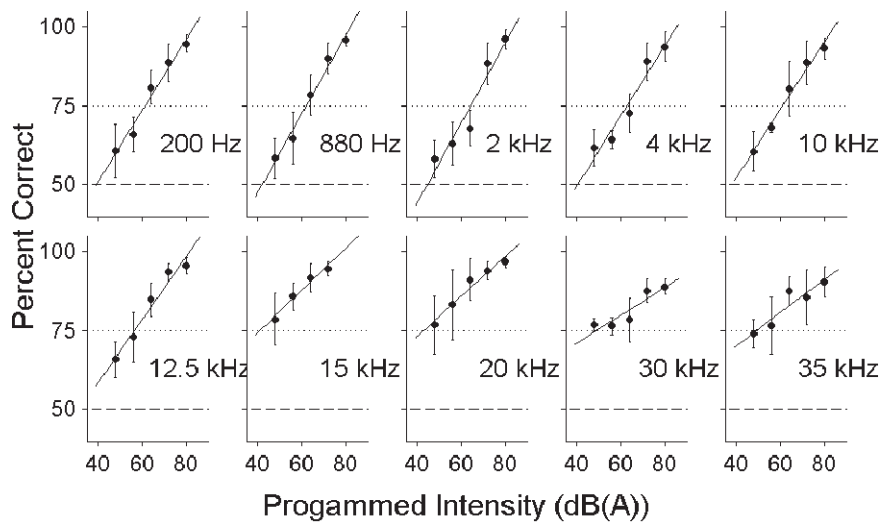


Fig. 4. The mean percent correct over all possums for each tone frequency and intensity [≥ 48 dB(A)] over all test sessions as a function of the programmed tone intensity [dB(A)]. The vertical lines indicate one standard deviation on each side of the data point based on the means of the individual data. The horizontal lines mark 50% and 75% correct. The straight lines were fitted to the data by the method of least squares. The slopes, intercepts, the standard errors of the fits (SE) and the percentage of variance each line accounted for (VAC%) are given in Table 2.

ly reducing tone intensity and examining the overall percent correct. Percentage correct is one accepted measure of sensory acuity in animal psychophysics (Blough & Blough, 1977). Here percentage correct decreased directly with tone intensity but did so to a different degree at different frequencies. It decreased more gradually at the higher frequencies (Figures 2 and 3 and Table 2). One problem with percentage correct is that, because of the method used here, it includes data from both tone-on and tone-off

trials and so precluded examination of performance on these two trial types separately. Thus the SDT measures of P(Hits) and P(FA), which show performance on the different trial types, were also examined (Figure 5). These showed similar degrees of change at the higher frequencies but asymmetric changes at the lower frequencies. White and Wixted (2010) also report asymmetric performance on a yes/no task with the asymmetry coming mainly from a constant FA rate. In the present data the asymmetry comes from the hit rate

Table 2

The slopes and intercepts of the lines fitted by the method of least squares to the overall means of the percent correct and d' at each intensity are shown for each frequency. The standard errors of the fits (SE) and the percentage of the variance in the data (VAC%) accounted for by these lines are also shown.

Frequency	Percent Correct				d'			
	Slope	Int.	SE	VAC%	Slope	Int.	SE	VAC%
200 Hz	1.13	5.72	2.36	97.3	-4.34	0.098	0.208	97.2
880 Hz	1.25	-2.43	2.17	98.1	-4.24	0.097	0.148	98.6
2 kHz	1.26	-6.14	4.36	93.1	-4.83	0.102	0.320	94.2
4 kHz	1.11	5.12	3.45	94.3	-3.90	0.090	0.264	94.9
10 kHz	1.07	9.46	1.98	97.9	-3.40	0.083	0.068	99.6
12.5 kHz	1.00	18.52	2.55	96.1	-3.00	0.080	0.229	95.1
15 kHz	0.67	47.19	1.31	96.6	-1.72	0.070	0.035	99.8
20 kHz	0.63	47.74	1.82	95.1	-1.75	0.066	0.082	99.1
30 kHz	0.44	53.30	2.44	83.9	-0.81	0.041	0.152	92.0
35 kHz	0.53	49.04	2.75	85.4	-1.11	0.048	0.197	90.4

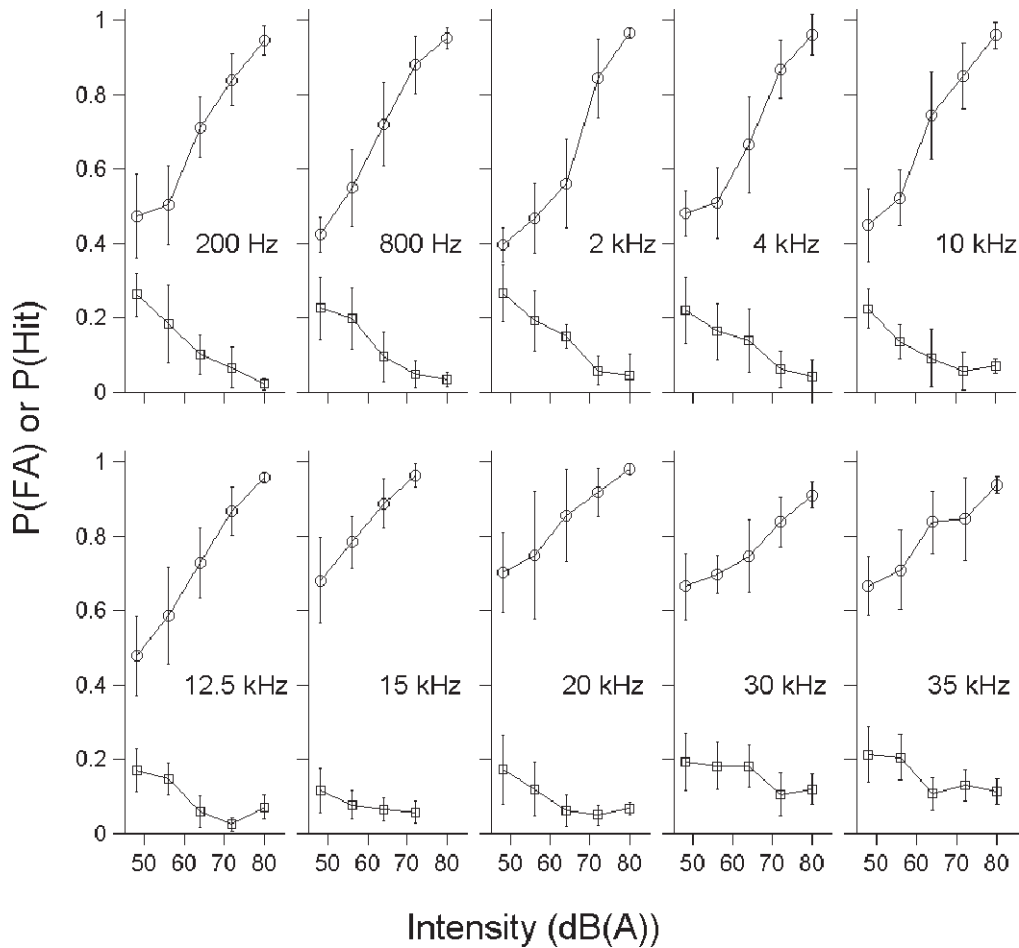


Fig. 5. The probability of an incorrect response on a tone-off trial [P(FA)–squares] and the probability of a correct response on a tone-on trial [P(Hit)–circles] for the overall mean data from all possums plotted against the intensity for each frequency for all intensities greater than or equal to 48 dB(A). The vertical lines indicate one standard deviation each side of the data point based on the means of the individual data.

increasing much more steeply than the FA rate decreases at the low frequencies, and FA rate is not constant. White and Wixted found symmetric performance on a two-alternative forced-choice task, similar to the finding at the higher frequencies here. Thus, unlike White and Wixted’s data, the degree of symmetry here was frequency-dependent and not task-dependent. The degree of asymmetry in the present data is related to asymmetric changes in response bias. If sensitivity changed as intensity decreased and there were no response bias these data would change symmetrically. At the training intensity, for all frequencies, the possums’ hit rates were high (around 90% or above) and FA rates very low

(often near zero), showing little response bias. At the lowest intensities there was a bias towards the “no” responses in both measures, but, at the low frequencies this was greater on tone-on trials than on tone-off trials. It is not clear why these auditory stimuli produced different results from the coloured stimuli used by White and Wixted. The measure of sensitivity based on the Hit and FA rate is d' . In the present study this measure changed similarly to percentage correct with intensity and frequency (Figure 6). The functions relating d' to intensity at each frequency were used to produce relative sensitivity functions using the method outlined by Wright (1974). Results indicated

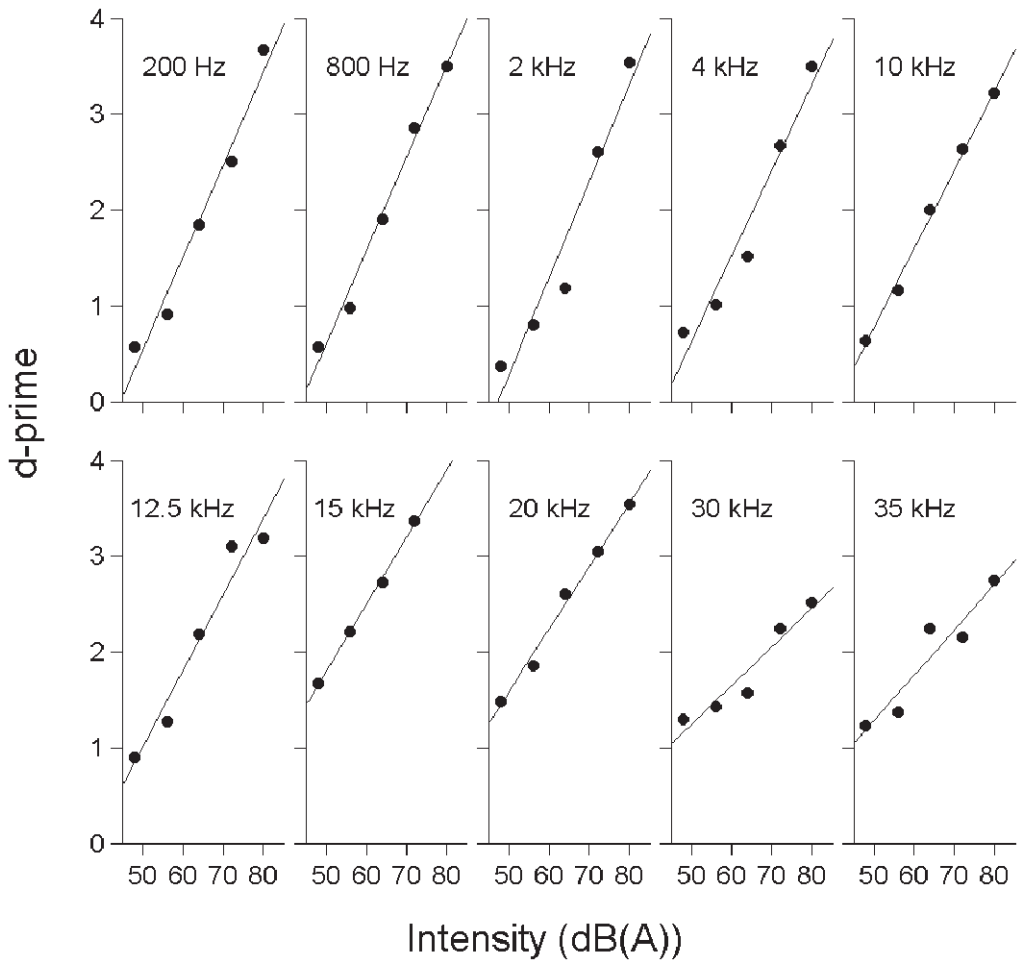


Fig. 6. The mean d' over all possums for each tone frequency and intensity [≥ 48 dB(A)] over all test sessions as a function of the programmed tone intensity [dB(A)]. The lines were fitted as in Figure 4 and parameter values and measures of fit are given in Table 2.

that in the testing context the 5 possums heard low intensity tones best but were still sensitive to tones of 20 kHz and above. This relation was observed for the animals as a group and for each individual. It should be noted that the maximal sensitivity was within the range of frequencies for which intensity could be assessed using the sound pressure meter. Sensitivity decreased between 15 and 20 kHz (both tones that were within the range of the sound pressure meter) and decreased further for the two high frequency tones where intensity was set by calibration. It was clear, however, that the possum could still hear these higher frequency tones.

Percent correct against intensity gives a psychophysical function and such functions are frequently ogival (Engen, 1972). Nonetheless, straight lines describe the present data very well. Likewise the relation between d' and intensity was well described by straight lines. The slopes of these lines reflect the degree to which sensitivity (measured by percent correct or d') to the sounds changed with intensity changes. The shallower the slope the less sensitivity changed as intensity decreased and the greater the difference between the equal d' contours in Figure 6. The shallowest slopes and the greatest differences between these contours are from the four highest frequen-

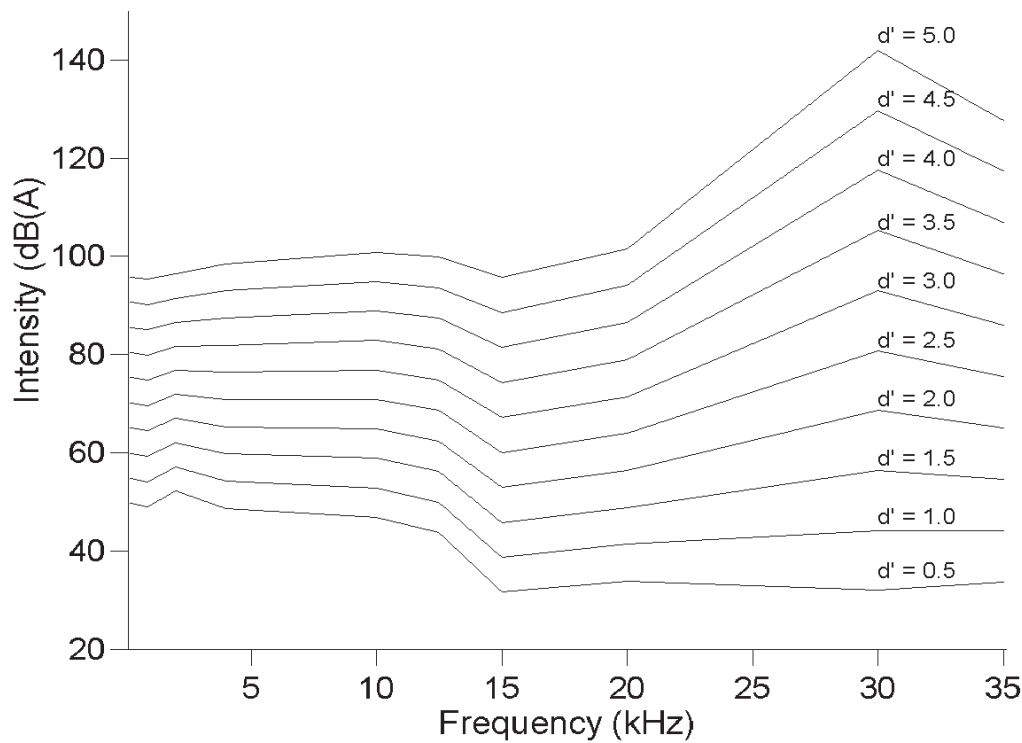


Fig. 7. The intensities equivalent to various d' values (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0) for each frequency (derived from the lines in Figure 6 and Table 2) plotted against frequency, with those from each d' value linked to show the equal d' contours. In some cases the values shown were interpolated from the psychometric functions.

cies. As Signal *et al.* (2001) pointed out, when a single measure of sensitivity is required, then the intensity related to 75% correct (or the 75% threshold) is often recommended as appropriate for use with two-choice discriminations such as the present one. Signal *et al.* (2001) found that this provided a somewhat higher threshold value than the electrophysiological data of Aitkin *et al.* (1979) and they argued for the use of the 50% threshold as indicating some ability of possums to detect the tone. The use of a 75% threshold as a measure of sensitivity is based on the assumption that accuracy on tone-on trials will reduce to 50% correct when the tone cannot be detected in that context but that on tone-off trials accuracy should stay high as these trials do not change in difficulty as the tone intensity changes. As mentioned earlier, Figure 6 shows that with frequencies below 15 kHz, detection accuracy on tone-on trials [i.e., the probability of detecting a tone or P(Hit)] decreased proportionally more than did the probability of reporting a tone present

on a tone-off trial [i.e., P (FA)]. These data support the use of a 75% threshold. However, these plots also show that with tones over 15 kHz, P(Hit) and P(False Alarm) decreased similarly—supporting the use of a 50% threshold.

It should be noted that in the present study, as in the research by Signal *et al.* (2001), auditory acuity was determined in the context of the background noise present in the home room. Signal *et al.* (2001) used the same colony room and equipment as in the present experiment and they replicated the whole testing procedure with one possum in a separate sound-attenuated chamber. Their Figure 4 shows that they found essentially no difference in the psychophysical functions with 880 Hz for this possum when the data from the sound-attenuated chamber and from the home room were compared. Thus, it appears that the background noise did not affect that possum's ability to detect that tone. The steady background noise intensity in the home room was around 28.5 dB(A) and it was cage rattling

that gave the occasional peak [to around 52 dB(A)] rather than a general noise intensity increase. Signal et al.'s (2001) data suggested that the background noise was not interfering with their measures with the 880 Hz tone. Nevertheless, because results may have differed at other frequencies, a conservative tack was taken in the present study and data were excluded from any trials where the dB(A) level was set below 48. Examination of the data in Figures 2 and 3 do not suggest any consistent differential variability at any intensity for any frequency. Thus it does not appear that the occasional overlap of the home room background noise with the 48 dB(A) tones made the data any more variable at that value than at the other intensities for any frequency.

Signal et al. (2001) provide the only prior published study of possums' hearing acuity. That study used procedures comparable to the present one to determine possums' ability to detect an 880 Hz tone. The results were very similar to the present results with that same frequency (their 50% threshold was between 29 and 46 dB while here this was 42 dB, and their 75% threshold was between 60 and 68 dB, here it was 62 dB). The orderliness and similarity of findings in the two studies suggest that the procedure used provides sensitive and reproducible behavioral indices of auditory acuity.

One difficulty with conducting the present study was the production and measurement of frequencies normally beyond the range of many humans' ability to hear (the normal human hearing range is 20 Hz to 20 kHz). Audio equipment is designed for use by humans and to move beyond this range poses a challenge for researchers. Low frequency sounds also pose a problem as they can produce resonance and such vibrations can be detected by senses other than hearing. Fortunately it is possible to measure sound pressure waves by capturing them with a microphone or tweeter and using an oscilloscope to assess the wave form and amplitude obtained. Prior to using any sound frequency in the present study the wave forms produced were examined and it was clear that many tones below 200 Hz resulted in resonance and so these were not used. Thus, the lower frequency end of possum hearing remains to be explored. The wave forms for the higher frequency tones were clear and the sound

production and control systems used appeared to reproduce them accurately; even the 30 and 35 kHz tones wave forms appeared to be appropriate. Unfortunately, no sound pressure meter available to us was scaled to measure the intensity of these two high frequency tones. Therefore, a calibration procedure was used for the two highest frequencies. This allowed the sound pressure meter to be used to check and, if necessary reset, the tone intensity each session. The calibration was checked against oscilloscope readings and appeared to be accurate. The programmable audio generator was specified to produce tones up to and including 35 kHz but no higher; therefore, this was the highest frequency we could test. It remains to be seen if possums can hear even higher frequencies. Finding devices that can produce and measure these sounds accurately is a real, but not insurmountable, problem.

Results obtained with the behavioral procedure in the present study suggest sensitivities similar to those obtained by Gates and Aitkin (1982), who conducted a microelectrode mapping survey of the auditory cortex of possums. They found that possums' hearing was relatively sensitive at frequencies between 330 Hz and 39 kHz, with the greatest sensitivity occurring between 17 and 19 kHz. Gates and Aitkin reported that, in general, the possums' hearing was most sensitive at approximately 18 kHz. Thus, the relative sensitivity function based on the behavioral data obtained in the present study and the audiograms based on their physiological data are much alike. It is interesting that behavioral measures of auditory sensitivity in the current study and in the study by Signal et al. (2001) closely paralleled the measures of sensitivity taken at the auditory cortex, because neurophysiological and behavioral measures can differ (Gates & Aitkin, 1982).

If fact, they do differ when the results of the present study are compared to those reported by Aitkin et al. (1979), who conducted a cochlear microphonic potential study. They found that the most sensitive hearing range for possums was between 800 Hz and 1 kHz. They found hearing sensitivity increased as the tone frequency increased from 100 to 800 Hz, and then decreased as the frequency increased from 1 kHz to 10 kHz, but no higher frequencies were examined. These results differ from those of the present study, but

the limited range of frequencies studied by Aitkin *et al.* (1979) limit conclusions. It does appear, however, that systematic examination of the correspondence between various measures of acuity merits investigation and is an area for further research.

Given the frequencies at which possums hear best, it also appears worthwhile to extend Winter's (1976) investigation of possum calls, which revealed calls ranging from 500 Hz to over 12 kHz. Winter was unable to record and measure frequencies beyond 12 kHz, but suggested that calls could reach frequencies above 12 kHz. Thus, a second area for further research would be to investigate possums' calls further, especially those in the higher frequencies. The present data suggest that possums could hear such calls well, increasing the probability that they would be observed.

The present study is limited in that the full range of frequencies that possums can detect was not determined. Both lower and higher frequencies remain to be studied. Interference prevented this study exploring frequencies lower than 200 Hz and equipment prevented the study going higher than 35 kHz. Thus a third area for future research is determining the lowest and highest frequencies that possums can detect reliably. Such information, like that of the present investigation, is useful in understanding the behavior of these animals and may be put to use in practical strategies for controlling the animals. For example, frequencies that possums detect especially well may be aversive at relatively low intensities and it may be possible to arrange sound barriers that prevent them from entering sensitive environments. Investigating this possibility is a fourth area of further investigation and one of considerable importance.

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